ENVIRONMENTAL STUDIES ON LEPTOSPIRAE IN HAWAI'I

C. H. Sengbusch
Lieutenant Commander, Medical Service Corps
U. S. Navy

Navy Environmental and Preventive Medicine
Unit Number Six
Pearl Harbor, Hawai'i

Many of Hawai'i's scenic freshwater streams and ponds contain bacteria of the genus Leptospira, of which two species have been proposed. The first, Leptospira biflexa, exists in the environment as a saprophyte and is of no medical importance. The second, however, Leptospira interrogans, is pathogenic to humans and produces the severe to moderate febrile illness known as leptospirosis. The benevolent climate of the Hawaiian Islands has fostered occupational and recreational activities which bring both the local populations and many visitors into contact with leptospire in the environment. As a result, the State of Hawaii has been reporting an annual incidence of 1.08 cases per 100,000 people (Martone & Kaufmann 1979), more than 20 times the national average. These factors, plus the difficulty in diagnosing the various manifestations of the disease either clinically or in the laboratory, prompted the Navy's Environmental and Preventive Medicine Unit Number Six (NEPMU-6) to investigate the extent to which these bacteria occur in selected environments. Three years' work in cooperation with the Hawaii State Department of Health has now been completed.

Leptospirosis Investigations

Three methods were utilized in the investigations to assess leptospire in the environment: (1) bacteriological culture of kidneys from rodents; (2) serological surveillance of human populations at risk; and (3) direct culture of surface water. The procedures followed the standard recommendations of the Center for Disease Control (Sulzer & Jones 1976). The investigations were designed with an awareness of some of the unique aspects of leptospirosis in the Hawaiian Islands. The State has many of the natural hosts of the disease, such as horses, pigs, and cattle, in addition to the commonly infected rodents. The latter includes several species of rats and mice throughout the Islands, plus the mongoose, a carnivore introduced into Hawai'i to control rats and snakes. Human infections occur when the leptospire are spread by the urine of infected animals into surface water utilized by man for agriculture or sports. In Hawai'i, taro fields are often implicated in leptospirosis cases, as well as swimming or wading in contaminated water. Military personnel are at the same risk of recreational exposure to the

disease as any native or visitor to the State, but they are also exposed during training exercises.

Midway Island, at the northwest end of the Hawaiian archipelago, was chosen for the first investigation because the large rodent population posed a possible threat to the water supply. Thirty rodents were kill-trapped and their kidneys removed for culture. The cultures were examined by Darkfield microscopy and the leptospire identified by their characteristic bend at one or both ends. The results (Table I) showed that 60% of the animals were infected, compared with a similar survey at Pearl Harbor where only 33% of the examined animals were infected.

TABLE I. Results of rodent cultures.

	Midway	Pearl Harbor
Number Tested	30	12
Number Positive by Darkfield	18	4
Percentage Positive	60	33

The second investigation utilized serological reactions in possible victims' blood to assess the extent of possible leptospirosis. Blood samples were obtained from random Navy, Marine Corps, Navy Public Works employees, and from civilians submitted by the Department of Health on Kaua'i. The blood samples were tested by macroscopic slide agglutination against pooled antisera. The results (Table II) indicated that individuals in the Marine Corps were receiving greater exposure to the bacteria than any of the other populations.

TABLE II. Results of serological testing.

Source	Total Tested	Number Positive	Percentage Positive
Navy	142	2	1.4
Marine Corps	24	3	12.5
PWC Workers	32	0	0.0
Kaua'i	269	1	0.4

Direct culture of surface water suspected of containing leptospire was employed in the third investigation. One hundred milliliter samples were pumped through an 0.45 micron membrane filter, then a 1.0 ml aliquot was inoculated into Ellinghausen, McCullough, Johnson, Harris (EMJH) medium. The characteristic ring growth near the top of the tube culture was examined by Darkfield microscopy for Leptospira. Samples were obtained from the Nā Pali coast of Kaua'i. The results revealed that leptospire were present in many of the streams of the area.

The data from these three investigations provided enough information to make general assessments of the risk of leptospirosis infections at each location. These evaluations are shown in Table III. Specific areas of Kaua'i probably have considerable risk, perhaps equalled or exceeded by Midway. Leeward O'ahu definitely had the least risk of exposure. These findings are consistent with other work that has been done in the State (Higa 1978).

TABLE III. Leptospirosis assessment.

Locality	Possibility of Infection
Midway	Little to Moderate
Leeward O'ahu	Little to None
Kaua'i	Moderate

SURVIVAL OF LEPTOSPIRE IN VARIOUS CONCENTRATIONS OF SEAWATER

Current work at NEPMU-6 is focusing on the determination of the range of salinity of seawater which leptospire can tolerate. Leptospire are known to be viable for long periods of time in freshwater and to die in seawater. However, little data are available on the survival of these bacteria in brackish water. This project is a joint effort with the Vector Control Branch, Department of Health, State of Hawaii. The information will be of value in planning the safe use of Hawai'i's surface water both for recreational enjoyment and for economic gain. Seawater tolerance is also a vital consideration to the State's growing prawn industry in protecting the health of their workers while cultivating a profitable crop.

Materials and Methods

Two serovars of Leptospira--L. australis, an interrogens-type, and L. patoc, a biflexa-type--were tested against five dilutions of seawater obtained from an area east of the entrance to Pearl Harbor. Using duplicate tubes, 5.0 ml of each dilution was inoculated with 0.5 ml of a five-day old pure culture grown in EMJH medium. The resulting suspensions yielded greater than six motile organisms per high power field. The tubes were stirred and incubated at 30°C. Beginning after 24 hours, the cultures were observed every two to four days under Darkfield. Results were expressed as the average number of motile organisms observed in five random fields. Distilled and tap water were used as controls. The experiment was duplicated in autoclaved seawater.

Results

The control tubes in both types of seawater demonstrated more than six motile organisms per high power field in excess of 35 days. The results of the experimental tubes are shown in Charts I and II. In both the natural and sterilized seawater, concentrations of 50% or greater killed the leptospire in three days or less. The more dilute solutions of natural seawater took as long as 15 days to cause the same effect. The sterilized seawater, however, in concentrations of 25% or less showed no inhibitory effect of either serovars. While both serovars gave similar results in the sterilized seawater, L. australis appeared to have a shorter survival time than L. patoc.

Discussion

Seawater clearly causes rapid loss of motility, followed by death in Leptospira. In freshwater, the bacteria can easily remain motile for as long as 35 days. The dramatic difference between the survival times in natural and sterilized seawater suggests the possibility that other indigenous bacteria in seawater are the major factor in the inability of the leptospire to survive. Further work is definitely warranted to confirm this finding and to provide additional descriptions. An on-going experiment is attempting to reproduce these results and to clarify some of the factors which are influencing the survival of the leptospire. Certainly one of the implications of the preliminary data is that seawater concentrations in prawn farms of at least 25% may be needed to significantly reduce the risk of leptospirosis infection.

Preventive Measures

The risk of leptospirosis can be minimized by four actions. First, since penetration is presumed to be through the skin, workers in known hazardous occupations can wear protective clothing such as boots and gloves. Second, the contaminated

waters must be identified and avoided. Third, the public must be educated on the disease, where it occurs, and how it can be prevented. Fourth, infected animals, particularly rodents, must be controlled in areas exposed to human populations. NEPMU-6 has attempted to implement these measures among the Navy and Marine Corps populations in the State, just as the Department of Health has in the civilian community. Hopefully, these measures, plus new techniques such as using dilute seawater as a control, will make Hawai'i healthier for natives, the military, and the many visitors.

LITERATURE CITED

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Survival Of LEPTOSPIRA In Sea Water

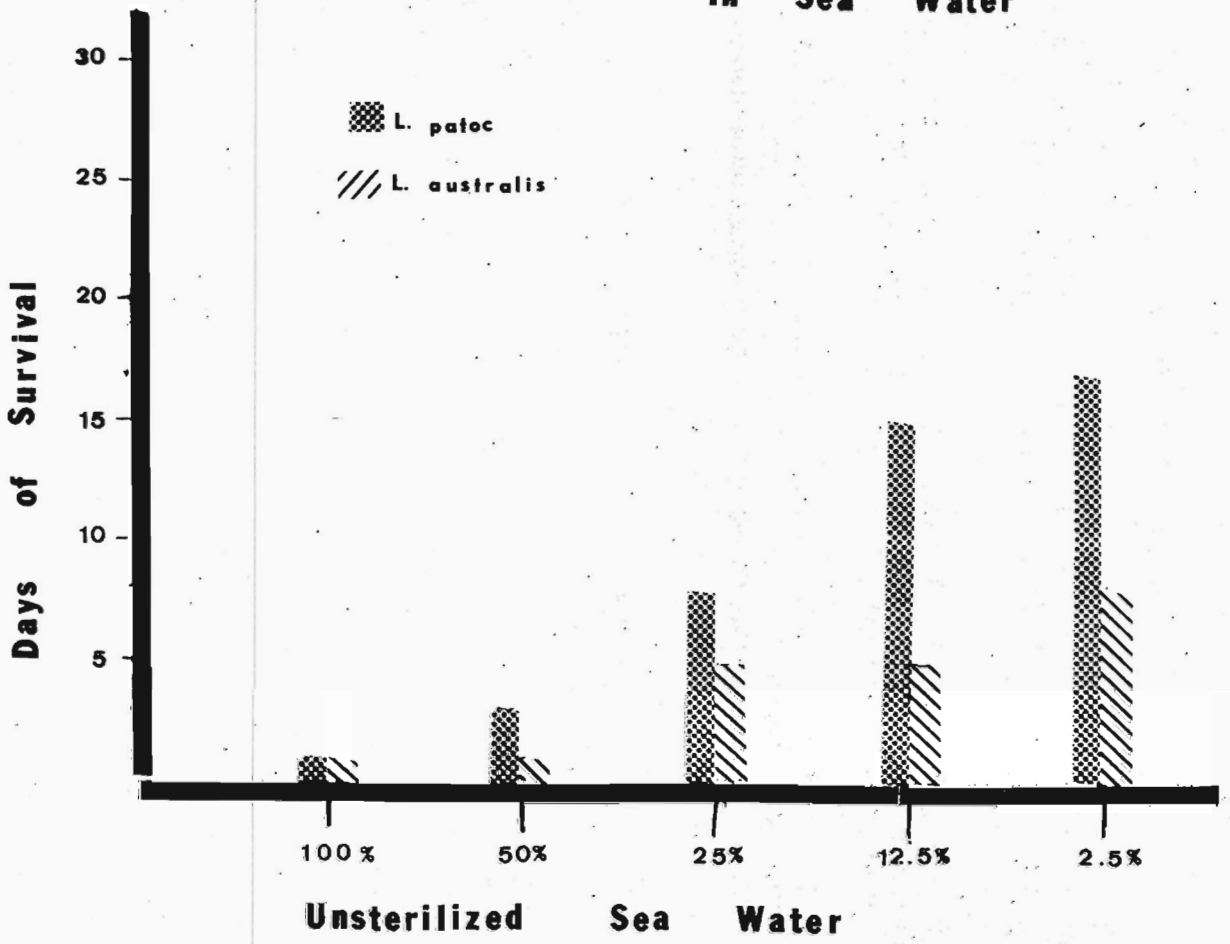

CHART I

NEPMU 6
1980

CHART II